CONTROLLED SUBSTANCES

ANALYTICAL METHODS

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Revision History

Revision #	Description of Changes
1	Original issue of Combined method: numbering change to fit new template, updating (clarifying) some procedures, and minor grammatical changes
2	Change to section #1: 4.1.2, 4.1.3.1, 4.1.5, 4.1.6, 4.1.7.2, 4.1.7.3, 4.1.7.4, 4.1.7.10, 4.2.1.1, 4.3, 4.4.1.5, 4.4.2.1, 4.4.3, 4.4.4, 4.5.3, 4.5.4, 4.5.5.5, 4.7.2.1, 4.7.3, 4.8, 4.8.1, 4.8.2 Change section #2: 4.1.1 Change section #3: 4.3.3, 4.5.1.2, 4.5.3.1, 4.6.4, added 4.7 Change section #4: 4.15 Change section #5: 4.1.1, 4.3.1, 4.3.2.1, 4.4.4 Change section #6: 4.1, 4.1.1.3, 4.1.2.5, 4.1.2.6, Change section #7: 1.2, 4.5.5.5 Change section #8: 1.3 Change section #11: 1.2
3	Changed section #1, 4.1.5, 4.1.7.3, 4.1.7.10, 4.2.2.1, 4.6.4, dropped 4.4.4, changed section #3, 4.3.4.3, 4.6.4, changed section #5 4.4.2, changed section #6, 4.1, 4.1.1.3, 4.1.2.2, 4.1.2.3, 4.1.2.5, changed section #7, 4.1.1.1, 4.1,2.4, 4.6.4.1, 4.6.4.2 added 4.6.3.3, changed section #8, 4.2.1.2, 4.2.1.3, deleted 3.6, 3.7 section #9, added 3.7, 3.8, 3.9, deleted 4.4.1, 4.4.2, 4.1.3, changed section #11, 4.1.3.1, 4.1.3.2
4	Changed section #1, 4.1, 4.1.1, 4.1.2, 4.1.7.2.1, 4.1.7.3, 4.2.2, 4.4.1.4, 4.4.3, 4.4.3.1, 4.7.2.1 section #2, 4.1.3, 4.4.1.4, section #3, 4.1.3, 4.5.4.1, 4.5.4.2 section #5, 2.3, 4.4.3, 4.4.4, 4.4.5, 4.4.6, section #7, 3.3, 3.4, 4.3, 4.4, 4.6.3.3 renumbered 4.6.3, section #8, 3.2, 3.5, 3.6, section #9, 4.2.1.2, 4.2.2 Added to section #1, 2.2, 4.6.4.2, section #8 4.2.2.1-6 and renumbered Deleted #1, 4.4.3.4

AM #1: General Quality Measures

1.0 Background/References

1.1 This analytical method contains guidelines describing how suspected controlled substance samples are to be analyzed, laboratory reports worded, what to do about analytical methods that are no longer or rarely used, sample and standards destruction, sampling rules, recipes for color reagents, identification criteria and operations of the GC/MS and the FTIR, and procedures for the analysis of controlled substances that require specific handling.

2.0 Scope

2.1 These methods/ procedures are specific for controlled substances but can also be used in identifying other drugs and some chemicals used in the manufacturing process. Analysis may be limited by the scope of ISPFS's accreditation, current instrumentation, and certain environmental conditions. These guidelines are a natural evolution of rules and procedures that have been used by ISPFS for years.

2.2 An infrequent test will be by definition one that has not been performed in a specific laboratory in over six (6) months. With the exception of Lysergic Acid Diethylamide (LSD), only analytes that are specifically listed with separate sections of this manual need to be tracked for frequency, i.e. iodine, phosphorus, psilocin, and GHB. Generally these analytes have distinct extraction schemes. When these tests are performed the positive controls must be analyzed before case samples. It will be up to each laboratory's management to decide how to monitor the frequency of these tests.

3.0 Equipment/Reagents

3.1 Not applicable

4.0 Procedure

4.1 Documentation in case notes

The electronic signature placed on each page of the examination data in the notes is the identification of the person responsible for the examination associated with the data, unless otherwise noted. When hands of the analyst is performed, the notes will clearly indicate who the trainee was and what they did. By submitting the case for technical review, the analyst acknowledges that the work done is theirs alone, unless otherwise noted.

All analysis will be performed on the date that the evidence was opened unless otherwise documented.

4.1.1 A description of the evidence, including all packaging and any leakage from internal packaging.

4.1.2 Physical description of evidence. Powder, liquid, plant material, etc. Including color, shape, additional residues, and any imprint or writing on pills/tablets/capsules, ampoules or manufacturers packaging. Documenting the description can be done by words, photos or drawings.

4.1.3 Original weight, number of pills, etc. of sample. See 4.1.5.

4.1.3.1 "Trace" or "residue" will be defined for solids as anything less than 0.10 grams, and for liquids as anything less than approximately 200ul.

4.1.3.2 All digits observed from a balance will be reported. For balances that normally read to the hundredths place, there may be instances where the sample weight is reported to the tenths place, in those cases the analyst will explain why in the case notes. This applies to reserve weights as well.

4.1.4 Amount used for analysis, or reserved weights need to be in the notes but do not need to be reported. Trace amounts of residue used do not need to be noted.

4.1.5 Exceptions; If the charge on a marijuana case is based on the number of plants, then the weight of the sample and the reserve does not need to be recorded. Weights and volumes of liquids are not to be reported. If more than half of the visible residue of a sample is to be consumed in analysis then any extracts must be returned with the evidence. Any extracts or washes from evidence that did not have visible residue will also be returned to evidence. Any extracts that had internal standard added to them, excluding methamphetamine quants, must be returned to evidence. This will be listed on the report. If the nature of the sample precludes an accurate weighing (sticky, sludge, moldy etc.) then the sample does not need to be weighed but the reason why must be reported.

4.1.6 If present in a sample a schedule 1 narcotic, LSD, or schedule 2 controlled substance will be confirmed if possible. If two or more CI narcotic, LSD, or CII controlled substances are detected in a sample, the component with the largest response must be reported. The presence of the minor component(s) need to be reported with a "indicates" statement. Their tentative identification needs to be documented but they do not need to be confirmed. Minor components with a response less than 10% relative to the major peak do not need to be reported or noted. Minor components that can reasonably be assumed to be a byproduct of the manufacturing process do not need to be noted either, examples include, but are not limited to, morphine and codeine in a heroin sample or p2p from a suspected clan lab.

4.1.7 Conclusions and Reporting

4.1.7.1 In the case of a sample that has a mixture of compounds from different schedules it is up to the analyst to determine if a schedule 1 non-narcotic and/or schedule 3-5 compound warrants confirmation. If it does not then the report should note that the sample indicates the presence of another controlled substance that was not confirmed.

4.1.7.2.1 If a drug is present but is unable to be confirmed, the report will read "indicates the presence of (name of compound), not confirmed". The reason why the drug was not confirmed must be on the report. This section does not apply to OTC type drugs, i.e. aspirin, caffeine etc.

4.1.7.2.2 If a controlled substance is present and could be confirmed but the analyst chooses not to, then the conclusion should read "indicates the presence of a controlled substance, not confirmed."

4.1.7.3 Non-analytical identifications of pharmecuticals will read "*source, including year*, if applicable, (PDR, Logo Index, Drugs.com, etc.) lists as "XXXX, schedule". Therapeutic ingredients other than the controlled substance will be noted in the case record if their presence affects scheduling.

4.1.7.4 All controlled substances should be scheduled. Exception; liquid samples in unmarked bottles or pills that have no observable logo/imprint that contain a controlled substance, where the schedule of the sample is dependent on the concentration of that controlled substance(s) should not be scheduled. If a liquid sample comes in a labeled pharmacy bottle or a pill has a recognizable logo and the results of analytical testing indicate the presence of the ingredients on the label, then any schedule associated with that label should be reported.

4.1.7.5 The reporting of non-controlled substances shall be left up to the discretion of the analyst.

4.1.7.6 In order to assist our customers with the conversion between metric and English units of measure on marijuana cases, the following statement can be added to the report: 3oz = 85.05g, 1lb = 453.6g

4.1.7.7 In order to report "No Controlled Substances detected", at a minimum, a sample must be run on the GC/MS using a temperature program and extraction scheme that encompasses a wide range of drugs. Refer to AM #3.

4.1.7.8 In multi-item samples, the report must clearly state what and how many items were tested, i.e. "Three white, oblong, imprinted M365 pills, analyzed one."

4.1.7.9 If a controlled substance is detected using an analytical scheme that is restricted to a limited range of compounds, the limitation does not need to be listed on the report. However, if the targeted compounds are not detected then that limitation must be clearly stated on the report along with any qualifiers. Example #1: "no basic drugs detected" qualifier: "examples of basic drugs include opiates, amphetamines and cocaine". Example #2, or "no psilocin/psilocybin detected".

4.1.7.10 If a wash sample/controls are run on a restricted scheme and the sample tests positive for drug XXX, and nothing is detected in the control, then for the control report "No XXX was detected" or add the qualifier (4.1.7.9).

4.1.7.11 If any drug is detected in the control sample, it must be reported.

4.1.7.12 For synthetic cannabinoids that have ambiguous scheduling report out "XXXX, a synthetic cannabinoid".

4.1.8 Records retention

4.1.8.1 Only the documentation used to reach the conclusion need be kept in the case file. These include chromatograms of sample(s), standard(s), library search results, blank(s), TLC plate photos, IR spectra, pill references etc. If a sample has to be reanalyzed, the reason must be in the notes.

4.1.8.2 Current batch documentation will be stored in an area of the laboratory known and accessible to the controlled substances chemists. An example of batch documentation is GC/MS autotunes.

4.1.8.3 GC/MS and FTIR data files will be backed up at least monthly.

4.2 Sample and Standard Destruction

4.2.1 Sample Destruction. For the purpose of this section a sample will be defined as any case work related extract, solution, or solid that is not returned to evidence. Standards of non-controlled substances will also be treated using these procedures.

4.2.1.1 Liquids are stored in waste bottles until disposal.

4.2.1.2 Disposal of aqueous liquids shall consist of neutralization of pH followed by solidification of remaining liquid with absorbent material (kitty litter etc.). The bottle and solid will then be discarded with normal trash.

4.2.1.3 Extracted plant material, test tubes, used vials, and TLC plates are placed in the disposable glass containers. Once these containers are full, they are stored until the next scheduled drug evidence burn, where they will be destroyed.

4.2.1.4 Solid (powder) samples can be either washed down the drain or placed in the liquid (aqueous) waste bottle.

4.2.2 Controlled Substance Standard Destruction. For the purpose of this section, a standard (primary, secondary, bench) is defined as any controlled substance used as a reference for confirmatory analysis.

4.2.2.1 When a controlled substance standard needs to be destroyed, i.e. past the expiration date, contamination, or degradation etc., then the standard will be stored until the next scheduled drug burn and destroyed then. Two lab personnel will witness the removal of the standards from the laboratory and fill out any necessary paperwork required by the agency conducting the drug burn. The laboratory standard log will indicate when the standard was destroyed. Any DEA forms will also be filled out and turned over to the proper authorities.

4.2.2.2 If a controlled substance standard is removed from the laboratory by being totally consumed, accidentally destroyed or spilled, the removal should be witnessed by a second scientist/FES and both individuals should sign and date the standard log.

4.3 Old Analytical Methods

There are numerous analytical or extraction methods that at one time were used in the Forensic Service laboratory system. These methods are not a part of this analytical method and are not to be used until they have been validated and approved.

4.4 Sample Selection

4.4.1 Sampling guidelines allow for the analysis of key evidence items within a case to maximize the resources of the lab. If during the pretrial process it becomes apparent that items not analyzed will require analysis, then upon resubmission that item may receive rush priority.

4.4.1.1 A felony charge has priority over a misdemeanor. Example: a gram of cocaine found in a suspect's pocket will be tested while a gram of marijuana found in the same pocket may not be.

4.4.1.2 A misdemeanor is treated equally to a felony if it is closer to the suspect or was the probable cause for a subsequent search. Example: A gram of marijuana found in a suspect's pocket would be analyzed in addition to a gram of cocaine found in the suspect's car.

4.4.1.3 Based on the analyst's training and experience if it is suspected different types of felony drugs are submitted, then one of each type will be analyzed. The analyst may use resources such as: statements of fact, description of items as well as visual inspection of items in making this determination.

4.4.1.4 The analyst will analyze evidence supporting the highest charge, i.e. trafficking, manufacturing, delivery vs. felony possession vs. misdemeanor possession.

4.4.1.5 When only a trace level of sample is present, every effort will be made to use less than one half of the sample. If it is necessary to use more than half of the sample, then any extracts, left over liquids, or residues will be returned with the evidence.

4.4.2 Multiple samples

4.4.2.1For less than trafficking amounts. The number of samples necessary to support the charge will be analyzed. Example: If there are five samples and the charge is possession, then only one sample needs to be tested. If the charge is intent to deliver, then more samples may need to be tested.

4.4.2.2 For trafficking amounts, **ALL** samples will be analyzed until the appropriate trafficking weight is reached. Example: Forty balloons come in, each with about 0.1g of suspected heroin. The analyst will weigh out enough to get to the first trafficking level, 2.0 g, and analyze each.

4.4.2.3 ONLY the results of the samples actually tested can be reported and testified to. No representation as to the content of the other samples is to be inferred.

4.4.3 Pharmaceuticals (pills, capsules, ampoules, etc.)

4.4.3.1 Pharmaceuticals need analytical confirmation if a literature search, or information provided by the agency, indicates that they contain a controlled substance, Schedule I or II. Exception, if a controlled substance has been analytically confirmed from a non-pharmaceutical sample in the case then a pharmaceutical (s) listed to contain the same controlled substance only needs a literature search, (section 4.1.7.3). If a literature search reveals that pharmaceutical with two, or more, different labels contain the same controlled substance then only one of the pharmaceutical needs to be analyzed. For the purpose of satisfying the "two test, two sampling" rule, described in 4.7.2.1, a literature search or a label from pharmaceutical packaging (ampoules, vials, blister packs, etc.) will be considered a presumptive test.

4.4.3.2 Acceptable literature references are published books (PDR, DIB, Logo index etc.), manufacturer's web sites, and Drugs.com. All literature searches shall be documented. Information from poison control centers and non-manufacturers' web sites can be used as a preliminary test when further analytical testing is performed or in conjunction with published books or approved websites to delineate pills with similar imprints and descriptions.

4.4.3.3 If an analyst, through training and experience, can make an educated assumption as to an identity of a partial pills content, that is subsequently analytically confirmed, then the results of a literature search of the partial pill can be used as a presumptive test. Examples of these type of pills are four part bar shaped Xanax, large four part Methadose 40, round Valium heart shaped center holes etc. For the purpose of this section only, if the contents of the partial pill yield a positive color test, then the results of the color test should be used instead of the literature search.

4.4.3.4

4.5 Color Test Reagents

Unless stated in another section of this analytical method, or below, the recipes for reagents found in "*Clarke's Analysis of Drugs and Poisons, 3rd edition*" are to be used.

4.5.1 The following list of color test reagents are approved for general use. Analyte specific color reagents may be listed in other sections of this method.

Marquis, Cobalt thiocyanate, Liebermann's, Mecke's, Froehde, Fast blue, Duquenois, Simon's (2° amines), Dille-Koppanyi, Sulfuric acid/UV.

4.5.2 The following reagents are approved as spray reagents Fast blue, Iodoplatinate, Van Urk (p-DMAB), Fluorescamine, and Dragendorff's.

4.5.3 For each reagent listed above, a worksheet recording the following will be maintained; reagents name, recipe, lot number and manufacturer of chemicals used if applicable, QC method, date made, name of preparer, and results of QC check. All reagents will be checked against known standards and a blank when they are prepared. Reagents that are prepared for one time use, i.e. Weber test, the QC results are to be documented in the case notes. If the effectiveness of a reagent is verified with each use and the results are documented in the appropriate case files, then no other documentation is required.

4.5.4 Shelf life. With the exception of Marquis, Cobalt thiocyanate, and Simon's, which are to be tested within 45 days prior to use, and Sulfuric acid when used for steroids , all reagents are to be tested with a positive control and a blank, or negative control as appropriate, with each use. Shelf life is thus considered indefinite.

4.5.5 The following reagents or situations require special attention;

4.5.5.1 Marquis: This reagent will degrade over time especially when not refrigerated. Test with both a positive (methamphetamine) and negative (dimethyl sulfone) control. When testing with methamphetamine, the reaction should flash orange immediately. If the orange reaction is slowed the reagent should be replaced.

The recipe for Marquis: slowly add 100mL sulfuric acid to 1mL of approximately 37% (w/w) formaldehyde.

4.5.5.2 Simon's (2° amines). Sodium nitroprusside stock solution "A" should be kept in the dark and refrigerated.

4.5.5.3 2% (w/v) cobalt thiocyanate aqueous solution. Mix cobalt thiocyanate with distilled/deionized water and filter if necessary. Solution should be clear and pink. A positive reaction produces a turquoise blue precipitate with cocaine. HCl is added to the test well containing the sample and cobalt thiocyanate if the sample is suspected of containing cocaine base. Test with both a positive (cocaine) and negative (dimethyl sulfone) control.

4.5.5.4 Fast Blue BB salt solution for marijuana and mushrooms. Add enough of the Fast Blue BB salt to distilled/deionized water to change the water to a yellow color. The exact concentration is not relevant as the solution is tested with each use and thus depends on the analyst's personal preference.

4.5.5.5 Duquenois. Add 2.5 mL acetaldehyde and 2 g vanillin to 100mL of approximately 95% or greater ethanol, may be denatured.

4.6 Authentication of Standards

Before a standard can be used as a reference for casework, it must be authenticated. This only has to be done once per lot.

4.6.1 Authentication is performed on the appropriate instrument, either a GC/MS or FTIR

4.6.2 A standard will be considered authenticated when the match (Q) is greater than 85%, as compared to a library search. If the match is less than 85% then two analysts must concur on the validity of the match. Initials of each analyst will be kept on the printout in the standards logbook or file. Reference libraries can come from any reliable source including but not limited to instrument libraries, scientific journals, or publications. When comparison to a journal, compendium or other document is not an option, mass spectral interpretation may be used in conjunction with the COA (certificate of analysis). This would apply in cases where instrumental data for a drug metabolite is not yet published, but a structurally similar compound is available to assist with interpretation. A second trained analyst must also review and initial the printout verifying the interpretation.

4.6.3 Authentication documentation will be kept for each standard.

4.6.3.1 Standards will be obtained from commercial or governmental sources i.e. Sigma, Cerilliant, Cayman, and DEA, etc. Standards may also be obtained from previously analyzed casework.

4.6.3.2 For qualitative analysis only, manufacturer's expiration dates may be ignored if the standard produces the expected mass/infrared spectra.

4.6.4 Unauthenticated reference material must be stored in a designated area or clearly marked that authentication is needed.

4.6.5 It is the responsibility of each analyst to verify that each standard or control used has been properly authenticated.

4.7 Identification Criteria

4.7.1 General Guidelines. The following identification criteria will be applied to both controlled and non-controlled substances unless different criteria are listed in another section of this Analytical Method.

4.7.2 Testing Rules

4.7.2.1 For each controlled substance, whenever possible, two positive tests from two different sampling events will be employed for identification. For confirmation one of the tests must provide structural information, i.e. either MS or FTIR. A positive test is defined as one that gives a reaction or result that indicates the presence of the analyte in question. Non-confirmatory tests include but are not limited to color tests, incomplete mass spectral data, retention time data, and a FTIR match to an external/internal library. If incomplete spectra are used as a test, then the reference used for comparison must be in the notes. A negative reaction to a color test cannot be used for a positive test even if a negative reaction was expected. Example: a negative reaction of methamphetamine and cobalt thiocyanate even though no color change is expected.

4.7.2.2 If only one sampling event can be performed on a sample, then n-tridecane internal standard is to be added to the extract before analysis on the GC/MS. The extract will be returned with the evidence. A blank with internal standard will also be run.

4.7.2.3For non-controlled substances i.e. inorganics, cutting agents and non-scheduled prescription drugs, the second sampling event does not have to be used.

4.7.3 If a sample's MS or FTIR spectra matches the spectra of a standard, the GC has a retention time within the acceptable time window, and the second test is positive, if ran, then the compound is confirmed.

4.8 Uncertainty of Measurement on Qualitative Samples

Of the many possible variables that contribute to the uncertainty of measurement using this Analytical Method, only one is accurately measurable, the use of a balance. For weights near regulatory limits, the contribution of that variable is what will be reported. It must be clear that that uncertainty number applies only to the act of weighing and not to the sample as a whole. All analysts must be aware of other possible variables and to be able to explain their potential impact on the reported weight.

Examples of other variables include but are not limited to, moisture/solvent content, static, and the ability to remove all of a sample from packaging. A set of sample pillows has been provided to each lab. These need to be weighed and the results recorded at the same time that the class 2 weight check is performed. Once a year the UM for the balances will be recalculated and any change will be published.

4.8.1 The uncertainty of measurement (UM) is; for less than 100g it is (+/-) 0.05g, for greater than 100g it is (+/-) 0.13g. For balances that only read to the tenths place the UM is (+/-) 0.3g

4.8.2 When the total weight of a sample(s) falls within the window of uncertainty at regulatory limits, then the uncertainty associated with each weighing event must be listed on the report. Current windows:

For Cocaine and Methamphetamine:

27.50 to 28.50g

198.70 to 201.30g

398.70 to 401.30g

For Heroin:

1.50 to 2.50g

6.50 to 7.50g

27.50 to 28.50g

For Marijuana:

84.55 to 85.55g (3 ounces)

452.30 to 454.90 (1 pound)

2266.70 to 2269.30g (5 pounds)

11338.70 to 11341.30g (25 pounds)

4.8.3 The measurement result shall include the measured quantity value (X) along with the associated expanded uncertainty (U), and this measurement shall be reported as X (+/-) U where U is consistent with the units of X, i.e. 28.05g (+/-) 0.05g. On the report (additional notes section) reference that the uncertainty was calculated at the 95% confidence level.

4.8.4 The total U is a product of the calculated uncertainty and the number of weighing events (samples weighed). For example:

0.25=0.05(5) for less than 100grams with five weighings.

AM #2: **FTIR**

1.0 Background/References

1.1 The Fourier Transform Infrared Spectrometer (FTIR) is an analytical instrument that is used to identify compounds based on their infrared absorption properties.

2.0 Scope

2.1 The FTIR is generally used with samples of higher purity. Samples may need cleanup prior to analysis.

2.2 The FTIR is primarily used to analyze organic compounds, compounds containing carbon atoms.

3.0 Equipment/Reagents

3.1 A FTIR and corresponding analytical software.

3.2 IR grade potassium bromide (KBr). Should be kept in a desiccator.

3.3 ACS grade solvents.

3.4 Hydraulic or other press for making KBr windows.

3.5 Any other sample introduction equipment, such as an ATR.

4.0 Procedure

4.1 Routine Maintenance

4.1.1 The desiccant in the instrument should be checked prior to running the monthly performance verification (4.1.2). If the desiccant is replaced it will be noted in the maintenance log.

4.1.2 Monthly performance verification. Using the manufacture's procedures, a performance verification of the instrument is done using polystyrene film. This procedure will be performed monthly and after any maintenance. The "System Validation Report" or "Valpro Qualification Report" printout is to be initialed by the analyst and kept in the maintenance logbook. If the verification does not pass and/or there is any other symptom of system failure, perform a bench alignment and repeat the verification and or consult the manufacturer. Any maintenance is recorded in the logbook.

4.1.3 The polystyrene "lollypop" ATR standard should be stored in a clean dry place. To preserve the film, a different section of the window should be used with each monthly check..

4.2 Background spectra will be collected immediately before every sample but do not need to be retained.

4.3 Standard Library Preparation

4.3.1 Production of valid standard library.

A pure sample of a standard is analyzed using the same procedures that will be used with an unknown (ATR vs. KBR etc.). Once a scan has been produced it can then be stored in an internal library. These standard scans can be produced and entered into the library as they are encountered in casework.

4.4 Analysis and Identification Criteria

4.4.1 Analysis

4.4.1.1 Run background

4.4.1.2 Obtain a spectra

4.4.1.3 Perform a library search of resulting spectra.

4.4.1.4 In order to confirm the presence of an analyte in a sample, the scan of the sample must match that of a known standard from the laboratory produced library. It is not acceptable to confirm on the basis of a match from a commercially produced library (Georgia State etc.). A match with an external library can be used as a preliminary (secondary) test.

4.4.2 Identification

4.4.2.1 FTIR spectra are considered matched if the peaks of the standard are present in the sample, in location, shape, and relative intensities. Any extra major peaks in the sample must be explainable.

4.4.2.2 If a sample's FTIR spectra matches a spectra of a standard and the second test is positive, then the compound is confirmed.

4.4.2.3 If FTIR spectral subtraction is done then what was subtracted needs to be in case notes.

4.4.2.4 If a FTIR spectra is inconclusive or negative for a controlled substance then the sample will be analyzed on a GC/MS.

AM #3: GC/MS

1.0 Background/References

1.1 The gas chromatograph mass spectrometer (GC/MS) is an analytical instrument that separates and identifies a wide variety of organic compounds based on their mass spectra and retention time data.

2.0 Scope

2.1 The purpose of this section is to layout the basic daily tune, scheduled periodic maintenance, sample preparation, and data interpretation necessary to perform quality analysis using a GC/MS. This method is limited to those compounds that produce adequate spectra and chromatography using the instruments owned and operated by the ISPFS.

3.0 Equipment/Reagents

3.1 A GC/MS and corresponding analytical software.

3.2 Capillary column and data acquisition methods sufficient to separate the analytes of interest.

3.3 ACS grade, or better, organic solvents.

3.4 Standards of the analytes of interest. Standard solutions may be prepared in-house or purchased from a commercial source. They can contain a single analyte or a mixture but all must be authenticated before use in casework.

3.5 Sodium carbonate and bicarbonate.

3.6 n-Tridecane internal standard. Use the ratio of 1.3ml to 1L chloroform.

4.0 Procedure

4.1 Mass Spectrometer Tune

4.1.1 Frequency

Using Hewlett-Packard/Agilent software and instrumentation an AUTOTUNE will be run after every major maintenance procedure, i.e. source cleaning or column change. They will also be run whenever a drift from expected values is encountered in the QUICKTUNE, see 4.1.2.

4.1.2 Using Hewlett-Packard/Agilent software and instrumentation, a successful MS QUICKTUNE or AUTOTUNE, will be run each day that the instrument is used. A day is defined as a twenty-four (24) hour period starting at the time of the tune. The exception to this is if the sequence of a methamphetamine quantitation run lasts longer than 24 hours.

4.1.2 Definition of a Successful Tune (using PFTBA)

Using Chemstation/Masshunter software the following parameters should be met.

4.1.2.1 Mass assignments within +/- 0.2 AMU of 69, 219, and 502

4.1.2.2 Peak widths (PW) should be within 0.1 AMU of 0.55.

4.1.2.3 The relative abundances should show 69 as the base peak, although it might switch with the 219 peak. Under no circumstances should the base peak be anything other than 69 or 219. The relative abundances should be anything greater than 30% for 219, anything higher than 1% for 502.

4.1.2.4 The Isotope mass assignments should be approximately 1 AMU greater than the parent peak and the ratios should be 0.5-1.5% for mass 70, 2-8% for mass 220, and 5-15% for mass 503.

4.1.2.5 The presence of mass 18 (water), 28 (nitrogen), and or 32 (oxygen) may indicate an air leak into the system. If any of these masses are above 10% relative abundance then maintenance to the instrumental system might be required. Although elevated levels indicate an issue, by themselves, they do not affect analysis other than possibly an elevated background noise level. Prolonged exposure to oxygen or water can lead to a decrease in the life of a column and thus should be addressed through maintenance.

4.1.3 The QUICKTUNE and AUTOTUNE printouts shall be initialed by a drug analyst and centrally stored.

4.2 GC/MS Quality Assurance

4.2.1 For each GC/MS, a standard containing at least one controlled substance will be analyzed on each day that samples are run. If for any reason this standard fails, change of retention time, MS scan, weak or no response etc., then a determination of the cause of the failure and corrective actions must be undertaken. Samples may need to be reanalyzed. Consultation with the discipline leader may be necessary. The failure of the standard due to instrument failure should be noted in the logbook, along with whatever maintenance that was performed to remedy the situation.

4.2.2 To confirm any substance, there must be a valid standard of that substance analyzed within twenty-four hours of the sample run, regardless of when the tune(s) were run.

4.2.3 If a sample is analyzed using a GC/MS acquisition method that is of limited scope and the results are negative or if there are indications of a second unrelated compound then the sample will be analyzed on the “DRUG” general screening method or a variant of the “DRUG” method.

4.3 General Scheduled Maintenance

All non-consumable items, that are repaired or replaced, must be entered into the maintenance logbook. Entrees into the logbook should include any symptoms of problems along with the status of the system after the repair has been completed.

4.3.1 Daily (consumables, i.e. items used once and then discarded). These items are needed to operate the GC/MS system but their replacement, or repair, does not need to be entered into the maintenance logbook.

4.3.2 Check and fill solvent rinse vials on autosampler, empty waste solvent vials.

4.3.3 At the start of the week before samples are run, and after maintenance, run a standard mix containing 200ug/ml each of methamphetamine (base), caffeine, cocaine, morphine, and alprazolam. The stock of this mix should be kept in a freezer and a few drops of it added to an insert equipped vial just before analysis. The response of each compound in the mix must have a signal to noise ratio of greater than five (5) before samples can be analyzed. The noise is measured (response difference between peak to valley) just before each analyte and ratioed to the corresponding peak's response. Peak response is measured from the base to the apex. Peaks do not have to be measured if it is obvious that their signal to noise ratio is greater than five. By comparing a test run to previous runs can help identify potential problems, i.e. loss of response, and the need for maintenance. An initialed printout of the TIC (Total Ion Chromatograph) is kept in the maintenance logbook. If a new column is installed, or the "DRUG" general screening temperature program is altered then a standard or set of standards will be analyzed consisting of at a minimum alprazolam, phentermine and methamphetamine. Alprazolam must be identified and phentermine and methamphetamine must be able to be separated by greater than 0.100 minutes, preferably to baseline, and identified. The TIC of these run/runs will be initialed by the analyst and kept in the maintenance logbook.

4.3.4 Annual

4.3.4.1 Replace solvent trap, and pump oil, if so equipped. Should be done when other maintenance is performed, approximately once a year.

4.3.4.2 Vacuum dust from electronics and fans.

4.3.4.3 If possible inspect and clean the autosampler guides.

4.4 Non-scheduled Maintenance

All non-scheduled maintenance is to be performed on an "as needed" basis as indicated from failure of the autotune, poor chromatography, and or other indications of a system failure. All of these types of repairs will be noted in the maintenance log.

4.4.1 Replace or trim column.

4.4.2 Clean MSD, replace filaments, gold seal, and injection liner, when needed. Consult with manufacturer's manual or software for cleaning procedure.

4.4.3 Replace electron multiplier if, after repeated cleaning of the source, the EM volt readings remain at or above 2500.

4.4.4 Replace any part, or system of parts, as necessary.

4.5 Data Interpretation and Conclusions

4.5.1 Retention time

4.5.1.1 A sample's retention time will be considered acceptable if a mass spectral scan of the analyte is within +/- 0.040 min of a matching scan from a known standard. Retention time window was determined using the method described in "EPA SW846, method 8000B, section 7.6, Revision 2, December 1996 ".

4.5.1.2 The instrumentation and data acquisition parameters must be sufficient to maintain a 0.100 minute retention time difference between analytes of interest that produce similar mass spectra. Positional, geometric and other isomers may be exempt from this requirement.

4.5.2 The analyte of interest's peak shape must be acceptable, i.e. limited tailing or fronting. *__Note__* Some compounds do not chromatograph well, i.e. stanozolol.

4.5.3 Mass spectral interpretation.

4.5.3.1 For the purpose of drug identification, analysis of mass spectra is one of pattern recognition. A great deal of the interpretation is dependent on each analyst's opinion as to what constitutes a match. All comparisons for the purpose of confirmation are made between analytical standards, not library searches, and the sample spectra. The following are the minimum requirements to determine a match.

4.5.3.2 Identification of the molecular (parent) ion, if normally present. * __Note__* Some compounds do not have molecular ions in their mass spectra.

4.5.3.3 Presence of the correct base ion. Exception, some compounds have several ions that depending on spectral shifting may change base ions, cocaine is an example of this. In these cases the base ion of the sample does not have to match that of the standard but does have to be present in significant abundance.

4.5.3.4 The ratios of the relative abundances of the major ions, from the sample, should be similar to those of the standard.

4.5.4 Conclusions

4.5.4.1 Confirmation. The retention time must be within 0.040 min of a valid scan of the standard and the MS spectra must match. If both conditions are satisfied and, if possible, the analysis of a second sampling event, then the GC/MS data may be used for confirming the presence of a compound.

4.5.4.2 Non-confirmation. If a drug is present but is unable to be confirmed, the report will read "indicates the presence of (name of compound), not confirmed". The reason why the drug was not confirmed must be on the report

4.5.4.3 If the RT or MS do not match, or there is no peak at all, then report, "No controlled substances detected".

4.5.4.4 As with all cases it is up to the analyst to decide whether or not to report non-controlled substances.

4.6 Blanks

4.6.1 The purpose of a blank is to check for carry-over between samples, and to verify the lack of contamination of the solvents.

4.6.2 Frequency. A blank will be run preceding each sample. If two extracts of the same sample are run consecutively then the sample blank only has to be run before the first extract.

4.6.3 Interpretation. A blank run is considered blank if an analyte(s) of interest would not be identified using the above criteria from 4.5.3.

4.6.4 If a blank has an identifiable analyte of interest, then the blank will be rerun to rule out contamination of the blank vs carryover. If it is determined that the blank is contaminated, then the sample(s) immediately following the suspect blank(s) will be reanalyzed after an acceptable blank has been generated. If the samples contain the same analyte found in the contaminated blank, then the samples must be re-extracted and reanalyzed.

4.7 "DRUG" method parameters:

Initial temp 80°C

Hold time 2 min

Ramp 10-30°C/min

Final temp 280-310°C

Final hold time 0-5 min

Carrier gas flow 1ml/min

Split ratio 100:1

Inlet temp 280+ °C

MSD transfer line 280-310°C

4.7.1 The analyst may vary GC/MS instrument parameters without performance verification other than a positive control. This includes changing the inlet temperature, temperature ramp and/or hold time, pressure program, injection volume and split ratio, and ion scan range. Acquisition parameters are stored with the data files.

4.7.2 When using GC/MS methods that are of a limited scope, i.e. LSD, GHB, Mushrooms etc. then a positive control must be run prior to the analysis of case samples to ensure that the analyte of interest can be detected.

AM #4 Balance Calibration Verification

1.0 Background/References

1.1 In order to ensure the integrity of the reported weights of controlled substances each laboratory within the ISPFS system maintains a set of weights that are used to verify the calibration of all balances and scales located in each laboratory

2.0 Scope

2.1 This section intentionally left blank

3.0 Equipment/Reagents

3.1 One set of ASTM Class 2, or better, weights. These weights must be NIST traceable and certified at the time of purchase. The documentation for the certification/calibration of the weights will be retained.

3.2 Top loading balances

4.0 Procedure

4.1 Verification

4.1.1 Within 45 days prior to use, each balance is to have its calibration checked (intermediate check) against a set of certified NIST traceable weights. Results are to be recorded in a log for future reference.

4.1.2 Each balance is checked using a set of ASTM weights as reference. This set should span the expected weights of samples that will be measured on each balance. An example: for the typical top loader 1g, 100g, and 2000g weights would be sufficient. The allowable deviation from the standard weights will be 0.01 g or 0.1%, whichever is greater.

4.1.3 Each laboratory will keep a log sheet for each balance in use. The log sheet will list the balance identification, the weights used, their indicated weight, whether or not the observed weight is within the tolerance of the balance, the analyst and the date on which the check was performed.

4.1.4 Once a year an independent vendor will calibrate each balance.

4.1.5 An independent vendor will calibrate each weight set once every five years. Upon return to the laboratory and before the weights are placed back into service the calibration will be verified. Calibration Certificates will be checked for compliance with ISO/IEC 17025:2005, and initialed.

4.1.6 The weights will be handled with gloves or tweezers to keep them clean. They will be transported and stored in their case.

4.2 Consequences

If a balance fails a calibration check, the check is repeated. If the balance still fails then it will be taken out of service until it can be recalibrated or repaired. The balance shall be tagged indicating that it is out of service.

AM #5 Methamphetamine Quantification

1.0 Background/References

1.1 Under normal circumstances quantification of a methamphetamine sample's purity is not part of the analytical scheme used by the Idaho State Police Forensic laboratories. By special request this analysis can be performed. This analysis will only be performed on casework in which a federal court has a stated interest. This analytical method was derived from the principles and methods detailed in EPA publication "SW-846" and the states of Oregon and Utah's quantitation analytical methods.

2.0 Scope

2.1 The following procedures have only been approved for the analysis of samples containing methamphetamine in a solid matrix. The Idaho State Police Laboratory reserves the right to reject any sample for quantitative analysis based on sample size or circumstance.

2.2 In order to minimize the largest potential source of error, samples that have a high moisture or solvent content may need special consideration.

2.3 At the laboratory's discretion all samples containing methamphetamine will be analyzed as a composite unless specifically requested by the prosecutor.

3.0 Equipment/Reagents

3.1 Gas Chromatograph/ Mass Spectrometer (GC/MS) and corresponding software.

3.2 Injector should have a split liner with a glass wool plug.

3.3 Solid methamphetamine hydrochloride. The purity is to be documented with a certificate of analysis from the vendor.

3.4 ACS grade chloroform stabilized with either ethanol or pentene.

3.5 Class A volumetric flasks.

3.6 1.0ml Gastight® type syringes. Syringes that are used to generate the standard calibration curve will have their accuracy checked before each use via section 4.4.7 of this method. The verification must encompass the expected working range of the syringe, 200ul and 800ul. Syringes that fail to meet the acceptance value of (+/-) 3% will be evaluated for accuracy and if necessary replaced. A syringe check is good for two weeks.

3.7 Internal standard: With a ratio of 1.3 ml of (98% or greater) n-tridecane per 1 L chloroform, prepare at least four liters per batch. Each sequence of samples and standards must be made with the same internal standard.

3.8 0.5N sodium carbonate solution.

3.8.1 Add 2.7g of sodium carbonate to 100mls of water.

4.0 Procedure

4.1 Generation of Standard Curve

A six point calibration curve will be generated.

4.1.1 Prepare a standard stock solution of 1,200 to 2,000 ug/ml. With an analytical balance weigh 30-50 mg of methamphetamine HCL salt, add to a 25ml volumetric flask and dissolve and bring to volume with the internal standard. Calculate the concentration.

4.1.2 Using the syringe, auto-sampler vials, and stock standard prepare an additional five 1.0 ml standards. Into five autosampler vials place 0.1, 0.2, 0.4, 0.6, 0.8 ml of stock standard and then dilute to 1.0 ml using the internal standard. The undiluted stock standard must be one of the points on the curve. If the stock standard point does not fall within the linear range of the instrument then a more dilute stock standard is prepared and a new curve is run or the acquisition parameters of the instrument can be altered, i.e. split ratio, and the original curve rerun.

4.1.3 Add approximately 100ul (3 drops) of a 0.5N sodium carbonate solution to each vial and mix.

4.1.4 Using the GC/MS software set up the calibration acquisition parameters and tables. The curve is to be generated using linear regression with the points weighted using the inverse square. For Agilent Chemstation/MassHunter software, the parameters and tables are found in the data analysis/calibration section.

4.2 Sample Preparation and Analysis

One of the basic requirements in determining an accurate quantification is that the sample must be homogenous. The sample must also be prepared using the same extraction procedure that was used in generating the standard curve. If a sample has been previously qualitatively analyzed and been resubmitted for quantitative analysis the sample must be reweighed before proceeding to 4.2.1. This new weight will be used in the final calculations. Contact with the discipline leader will be documented on samples that exhibit an abnormally high level of moisture or solvent.

4.2.1 Initially rough grind the sample with a mortar and pestle until the entire sample will pass through a US No. 4 sieve. Roll and quarter the sample until a representative sub sample of about 10 grams is obtained. Grind the sub sample until a fine powder is formed. ***Note*:** If the sample is less than 10 grams then grind the entire sample into a fine powder.

4.2.2 Using an analytical balance that is accurate to at least 0.1 milligram, accurately weigh out an amount of sample that is equal to, or less than, what was used for the stock standard, and place into a 25 ml volumetric flask. Add internal standard, dissolve, and bring to volume with internal standard. See 4.3.1.

4.2.3 Into an auto sampler vial aliquot approximately one milliliter of sample extract, add approximately 100ul (3 drops) of 0.5 N Na_2CO_3, mix and analyze.

4.2.4 Samples are to be run in duplicate (two separate weighing's and extractions). The results are averaged before being used for calculating the final result. The duplicate results must have a Relative Percent Difference (RPD) (labeled "differential" in the BEAST LIMS) of less than (+/-) 10%, if they are not then either first rerun the extracts or proceed to extracting a new pair of samples and analyze.

$$RPD = \frac{(R1-R2) * 100}{A}$$

Where R1 = Result of first run in percent

R2 = Result of second run in percent

A = Average of R1 and R2 in percent

4.2.5 If a sample(s) is to be forwarded to another laboratory for quantitative analysis, the originating laboratory will analyze the sample(s) qualitatively; prepare the sample(s) as per 4.2.1 above and then send a maximum of 1g per sample to the laboratory doing the quantitative analysis. If the original sample is less than 5 grams then the original sample can be sent without preparation.

4.2.6 The samples will be composited at the originating laboratory by mixing all of the samples that tested positive qualitatively for methamphetamine and the resultant mixture will then be processed per section 4.2.1.

4.3 *Calculation and Reporting of Final Results*

4.3.1 Calculation

Using Agilent software calculate the concentration in the vial (the computer software should do this). Use the following equation to calculate the concentration of the analyte in the original sample. All calculations may be done by hand or by using computer software:

$$\frac{(\mathbf{A}\ \text{ug/ml}) \times (\mathbf{Milliliters\ of\ solvent})}{(10) \times (\mathbf{B}\ \text{mg})} = \mathbf{C}\ \%\ \text{analyte}$$

A = Concentration given by curve

B = Weight of sample used, in milligrams

If C is less than 20% then the sample is re-extracted and reanalyzed using a larger sample size. The calculated concentration of the re-extraction must be 100< C <20%.

4.3.2 Reporting

4.3.2.1 Using the formula:

$$\frac{C \times D}{100} = X$$

- Where **C=** average of the two duplicate results from the equation in 4.3.1
- (If the average is greater than 100% the results will be calculated using C= 100%)
- **D=** total weight of sample in grams

The uncertainty range of each sample will be reported out by weight using:

X (+/-0.07) = Range

Report the result that "All samples calculated as the hydrochloride salt." Each report will have the statement, "The expanded uncertainty value was calculated at the 95% confidence level".

4.3.2.2 If a sample is less than 0.5 grams the sample will not be quantitated and the report shall state "sample is unsuitable for quantitative analysis, insufficient amount". These samples will be qualitatively analyzed.

4.3.2.3 If the concentration of a sample is below 10% then the report shall state, "Sample is unsuitable for quantitative analysis, concentration is below the limit of quantitation".

4.3.2.4 All calculated results will be reported to the same degree of significance.

4.3.2.5 All results will be rounded using standard rounding rules, i.e. 1-4 down, 6-9 up and 5 to the nearest even number.

4.4 Notes and QA/QC

4.4.1 The curve must be linear as defined by a correlation coefficient of 0.998 or better. The correlation coefficient is generated by the Agilent Chemstation/MassHunter software.

4.4.2 The area counts of the internal standard should be consistent from the beginning to the end of the run (+/- 10% of the mean). If a sample's internal standard falls outside of the range then the sample can be added to the end of the sequence and rerun. If the drift is noticed after the sequence has finished then the sample needs to be reanalyzed with the next batch.

4.4.3 A new curve will be generated before each quantitation sequence. A sequence is defined as a batch(s) run consecutively without the introduction of non-quantitation samples. A batch is defined as up to twenty injections. At the end of each batch a positive control will be run, the results of which must be (+/-) 4% of the stated value. The Relative Percent Difference (RPD) will be calculated for each batch of positive controls:

$$RPD = \frac{|R1-R2|}{E} * 100$$

- Where R1 = calculated result of the first positive control run after the generation of the curve.
- Where R2 = calculated result of positive control run at the end of the batch, or sequence if two or more batches are run together.
- Where E = Expected value

The RPD will be less than 8%.

4.4.4 A positive control will be analyzed each time a curve is generated. The positive control will come from a source other than what was used to generate the curve. Another in-house standard from a different lot, if available, and prepared by a different analyst is to be used as the positive control. To a 25ml volumetric flask, add approximately 0.025g of methamphetamine hydrochloride salt, that has been accurately weighed using an analytical balance, then dissolve and bring to volume with internal standard. The positive control is made with the same batch of internal standard as the rest of the run. Aliquot one milliliter into an auto-sampler vial and add sodium carbonate solution.

4.4.5 The accuracy of the curve is validated when the value of the positive control is within (+/-) 4% of the stated value.

4.4.6 The calibration curve, chromatograms and quantitation reports of the positive controls, excel spreadsheets containing RPD (positive controls) calculation results and syringe verification results for each run, and sequence logs will be centrally located on the common forensics drive. Chromatogram(s) and quantitation report(s) of all samples, and chromatograms of all applicable blanks are to be kept in the case notes. Chromatograms of standards used to generate the curve do not need to be kept.

4.4.7 For the 1.0 ml syringe weigh 10 replicate aliquots of water at 200ul and 800ul. For the purpose of the calculations, the density of water is 0.998 g/ml. The acceptance criteria are (+/-) 3% of 0.1996 (for 200ul) and 0.7984 (for 800ul) for all measurements.

4.5 Uncertainty of Measurement

The (+/-) 7% value was derived from a validation study that took into account this method, different instruments and analysts. As long as the constraints described in this method are followed, the stated UM value is valid. Before a new analyst or instrument is used on casework it must be demonstrated that they/ it can meet the original target goals of the validation study. The UM value will be reviewed annually and adjusted if necessary.

AM #6 Analysis of Solid and Liquid Unknowns

1.0 Background/References

1.1 These procedures are designed to analyze most solid and liquid samples. Whenever possible, two different tests, and two different sampling events will be employed in confirming the presence of controlled substances. One of the tests must provide structural information, i.e. either MS or FTIR

2.0 Scope

2.1 Generally samples submitted to the laboratory fall into two categories, the first and most common, through information provided with the case or through training and experience the analyst has some idea of what the sample might contain. The second are complete unknowns. Analysis of some special/unique samples are covered under different sections of this manual.

3.0 Equipment/Reagents

3.1 The following pieces of equipment and reagents can be used in any combination to identify the analytes of interest.

3.1.1 A GC/MS and appropriate analytical software. Reference AM #3.

3.1.2 FTIR and appropriate analytical software. Reference AM #2.

3.1.3 ACS grade, or better, solvents.

3.1.4 0-14 pH paper.

3.1.5 n-Tridecane internal standard. In the ratio of 1.3ml tridecane into 1 L chloroform.

4.0 Procedure

4.1 Sample Preparation and Analysis

This section details the minimum requirements for analysis, any additional testing if necessary may be performed at the analyst's discretion. As per AM#1 section 4.8.2.1 a positive color test can always be used in lieu of a second GC/MS run or in conjunction with the FTIR in order to satisfy the second sampling event rule. This applies to both solid and liquid samples. While performing testing using two GC/MS sampling events, if the first GC/MS run is negative the second sampling event does not need to be analyzed. Any extract with internal standard must be returned with the evidence along with any unextracted sample. Liquid samples approximately as viscous as honey, or thicker, may be treated as a solid. Trace amounts of liquids may be treated as a solid or as a liquid at the analysts' discretion.

4.1.1 Solids

4.1.1.1 Weight. If possible obtain the weight of the sample. Packaging is never included in the reported weight unless the sample cannot be separated. In that case the report will clearly describe that packaging was included in the reported weight. On very rare occasions the submitting agency can request that a weight of the packaging, separate from the substance, be reported.

4.1.1.2 Perform screening tests.

4.1.1.3 Extractions. Acidic, basic, neutral, and dry extractions, in any combination, can be employed in order to separate diluents or other interferences, or improve chromatography. If screening tests are negative a dry extraction using methanol or a mixture of methanol and chloroform should be employed. Consideration of solubility's of the different analytes into various solvents must always be considered.

4.1.1.4 Analysis. Selection of appropriate instrumentation for confirmation is at the analyst's discretion, see methods 2 and 3.

4.1.2 Liquids

4.1.2.1 Option A: If possible, determine if the sample is aqueous, this may be done through (immiscibility or flame) testing (preferred) or from information submitted with the sample. If the sample is organic go to 4.1.2.5. If it is not possible to determine if the sample is aqueous then treat the sample as if it is. If submitted, a control can be used to determine if the sample is aqueous, they are assumed to be of the same matrix.

4.1.2.2 If possible, split the sample and perform a basic extraction using approximately 0.5N sodium carbonate or 0.5N sodium bicarbonate and an immiscible solvent, chloroform preferred. If less than half of the sample can be reserved and there was not a positive color test, add internal standard to the solvent before analysis on the GC/MS.

4.1.2.3 Option B: an aliquot may be evaporated with air/nitrogen and the resultant residue can be treated as a solid. A positive test on a second sample or the use of an internal standard on a GC/MS run is required for confirmation.

4.1.2.4 Refer to AM #1 section 4.1.7 for reporting conclusions.

4.1.2.5 Organic solvents may be analyzed directly with the GC/MS. Split the sample if possible and add internal standard if necessary. In order to produce adequate chromatography the sample may be diluted with appropriate solvent and/or base may be added before or after initial analysis.

4.1.2.6 Meth lab samples may be cleaned up using back extractions and solvent exchange.

4.1.2.7 Oil or glycerin based samples (steroids, e-cig etc.) may be diluted with an appropriate solvent or another clean-up method may be used.

4.1.2.8 If a sample is suspected of containing a controlled substance that is covered by a separate section, i.e. GHB, then that method should be used.

4.1.3 Analysis

4.1.3.1 Run samples using an appropriate data acquisition method, like "DRUG".

4.1.3.2 If a peak appears, and is not recognized, perform a library search.

4.1.3.3 If a controlled substance is recognized from a library search or other means, then a standard is run if identity is to be confirmed. Library search reports do not need to be retained in the case file.

4.1.4 Conclusions: see AM #3 section 4.5.4.

4.2 FTIR Sample Preparation and Analysis

4.2.1 Direct.

Solid samples or dried liquid samples may be analyzed directly with the ATR. Samples may also be mixed with KBr, pressed into a pellet/window and then analyzed.

4.2.2 Extractions

4.2.2.1 The organic layer from either a basic or acidic extraction may be mixed with ground KBr, evaporated and analyzed.

4.2.2.2 Samples undergoing a basic extraction may require bubbling with HCl gas and filtering before HCl salt can be isolated and analyzed.

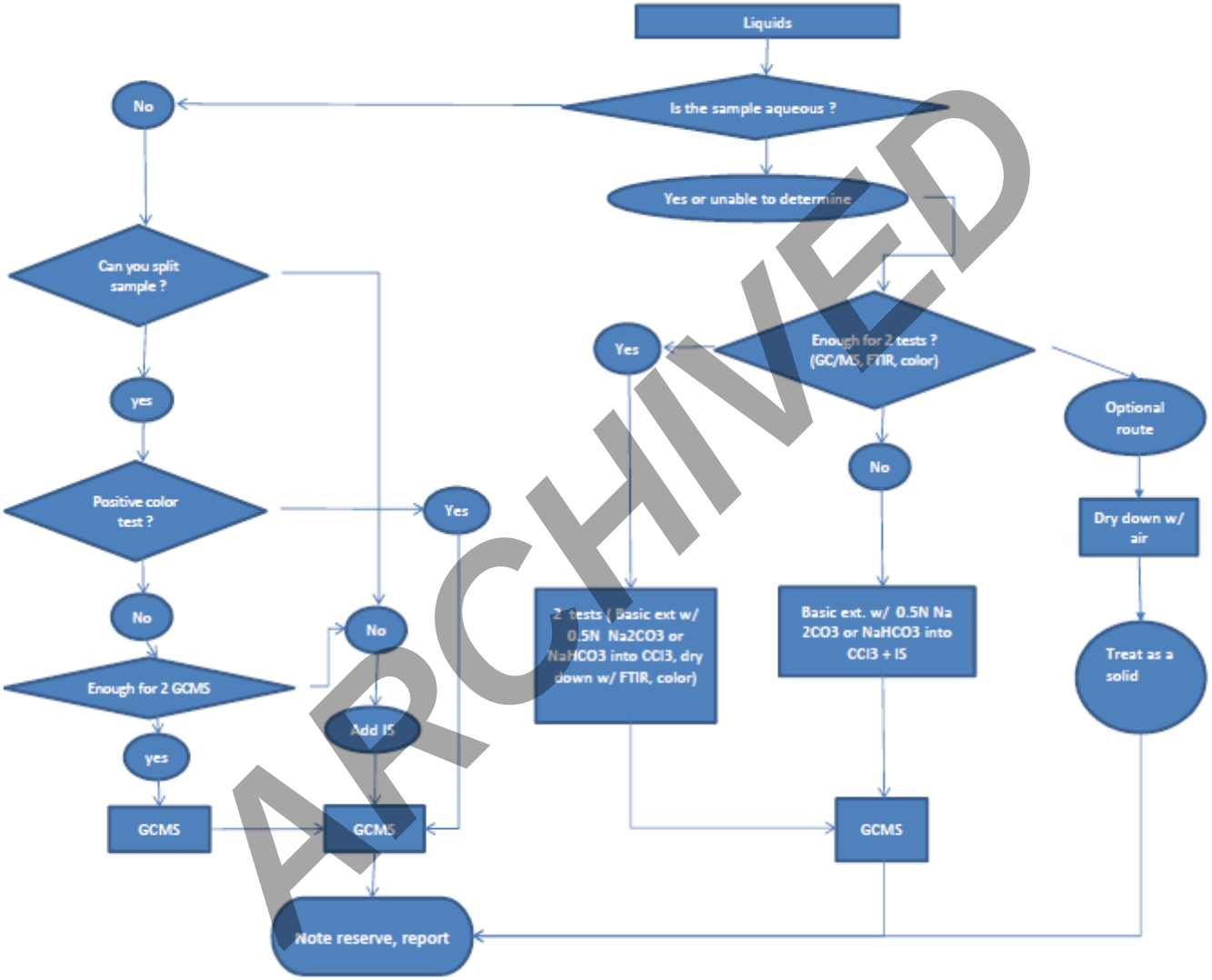
4.2.3 Analysis

See AM #2, section 4.4.1

4.2.4 Identification

See AM #2, section 4.4.2

5.0 Work Instructions



AM #7 Marijuana

1.0 Background/References

1.1 Marijuana (Cannabis Sativa) has been used for its sedative, euphoriant and hallucinogenic properties for over 3000 years. Written references to it date back to 2700 BC. It is primarily smoked but can be taken orally. The active compound, delta-9-tetrahydrocannabinol (THC) is most concentrated in the resin that is obtained from the flowers of the female plant. It is imperative that the analyst be familiar with the current Idaho code as it pertains to the legal definition of marijuana.

1.2 References

Identification of Marijuana*, by J.I. Thornton and G.R. Nakamura* Journal Forensic Science (1972), 12, 461

2.0 Scope

2.1 The following analytical procedures are used to confirm the presence of marijuana in plant material, residues, and samples containing extracted resins. The procedure is composed of a series of tests, none of which by themselves are specific for marijuana or THC, but taken in combination are considered specific for the presence of marijuana or its resins. GC/MS is not routinely applied to marijuana analysis but may be used and is considered specific for THC. If a plant material sample is suspected of containing a substance other than marijuana then the sample may be extracted and analyzed using a GC/MS.

3.0 Equipment/Reagents

3.1 Stereo microscope

3.2 Thin layer chromatography tank and plates.

3.3 Aqueous Fast Blue BB solution.

3.4 ACS grade Petroleum ether, hexane, diethyl ether, methanol, toluene, acetonitrile, and chloroform. Solvents may be used past any expiration dates listed by the manufacturer, since they are checked with a positive and negative control with each batch/use to demonstrate their reliability.

3.5 GC/MS and analytical software, see AM #3.

4.0 Procedure

4.1 *Solvent Extraction*

4.1.1 Plant material

4.1.1.1 Place some plant material in a test tube.

4.1.1.2 Cover with appropriate solvent.

4.1.1.3 Use extract for thin layer and/or modified Duquenois-Levine, see 4.3 and 4.4

4.1.1.4 Retain small amount of unused solvent as blank.

4.1.2 Resins

4.1.2.1 Flush/swab/extract item(s) containing suspected resins with appropriate solvent and collect solvent in test tube.

4.1.2.2 Use extract for analysis.

4.1.2.3 Retain small amount of unused solvent as blank.

4.1.2.4 For samples other than those that have been obviously heated, i,e, pipes, e-cig liquids, baked goods etc., their extracts should not be exposed to heat before analysis.

4.2 Microscopic Examination

4.2.1 Plant material is examined using a stereo microscope for the following characteristics:

4.2.1.1 Cystoliths and/or Cystolithic hairs – Small "bear claw" shaped hairs with bases of calcium carbonate. The cystoliths and hairs are located on the topside of the leaf or leaf fragment.

4.2.1.2 Unicellular hairs – Fine hairs located on the underside of the leaf or leaf fragment. **Note** Unicellular hairs are not always observed on the leaves from the budding parts of the marijuana plant.

4.2.2 Seeds are examined using a stereo microscope for the following characteristics:

4.2.2.1 Veined shell.

4.2.2.2 Ridged edges.

4.2.2.3 Point on one end and dint on the end of plant attachment

4.3 Thin Layer Chromatography

The extract used for the TLC should not be heated.

4.3.1 Spot a small amount of solvent extract onto a thin layer plate alongside of a marijuana standard and a solvent blank.

4.3.2 Develop the plate using one or more of the following mobile phases:

Hexane/diethyl ether 4:1 (petroleum ether may be substituted for hexane).

Chloroform or Toluene.

4.2.3 Visualize by spraying the plate with Fast Blue BB salt solution.

4.2.4 Compare results of unknown to those of standard, see 4.5.2. Photograph the plate for the case file.

4.4 Modified Duquenois-Levine

Unless otherwise noted the Duquenois-Levine test will be performed on the same day noted on the TLC plate.

4.4.1 In a test tube containing a portion of the evaporated solvent extract, mix 2-10 drops of Duquenois reagent and an equal amount of concentrated HCl.

4.4.2 Let stand ½ to 3 minutes and observe color change.

4.4.3 Add chloroform.

4.4.4 Observe if the purple/blue color transfers into chloroform layer. * **Note**: Transferring the solution from step 4.4.1 into a clean test tube before the addition of chloroform will decrease the color interference from chlorophyll.

4.4.5 A blank and a standard need to be run with each batch and the results recorded in the case notes.

4.5 Results and Reporting

A positive test shall be defined as the following:

4.5.1 Microscopic

Observation of cystolithic hairs on the leaf and/or the presence of characteristic seeds.

4.5.2 Thin Layer

Presence of a red spot with migration distance consistent with the red THC spot of the standard. The line of spots across the TLC plate should form a continuum, be it linear (straight line) or parabolic (slight curve with the ends being higher than the middle).

Negative blank.

4.5.3 Modified Duquenois-Levine

A purple* color developing after the addition of the HCl (*color may vary from blue to reddish purple depending on the sample).

Transfer of the color into the organic layer after the addition of chloroform.

4.5.4 A positive result shall be defined as the following:

4.5.4.1 Positive microscopic, single TLC system, and modified Duquenois-Levine.

Report using the words "marijuana or the resins thereof, (CI)".

4.5.4.2 Negative microscopic. Positive modified Duquenois-Levine and two positive TLC systems. The conclusion should contain the words "marijuana or the resins thereof, (CI)."

For positive GC/MS see 4.6.4.

4.5.5 Germination

Marijuana seeds without THC are only controlled if they are fertile. The germination test should only be performed if it has been determined that the seeds do not contain THC. ***Note*** In determining the presence of THC, soaking the seeds for up to thirty minutes in petroleum ether/hexane, does not affect germination rates.

4.5.5.1 Wrap a minimum of 10, to a maximum of 100 seeds, in a moist paper towel and place in a covered container. The container is then placed in a safe dark place for 14 days.

4.5.5.2 Check seeds daily making sure they do not dry out. Also watch out for mold.

4.5.5.3 Report how many fertile marijuana seeds sprouted as a percentage of the original total. Conclusion should be, "The sample contains XX % viable seeds with the botanical characteristics of marijuana seeds".

4.6 GC/MS Confirmation

4.6.1 Extract sample with appropriate solvent.

4.6.2 Run extract according to AM #3 along with a THC standard.

4.6.3 Compare retention time and ion chromatograph of sample with THC standard.

4.6.3.1 Report positive results as "marijuana or the resins thereof, (CI)" if a positive microscopic result was also observed.

4.6.3.2 Report “tetrahydrocannabinol, (CI)” if a positive GC/MS and positive TLC are observed.

4.6.3.3 Report ”tetrahydrocannabinol and/or tetrahydrocannabinolic acid” where there are two positive GC/MS analyses or if there is only one GC/MS result due to limited sample size, or a positive GC/MS and duquenois .

AM #8 Psilocyn/Psilocybin Mushrooms

1.0 Background/References

1.1 Psilocyn and psilocybin are related tryptamines that are found in many species of mushrooms. The mushrooms have been used in religious ceremonies for at least 3000 years by the native peoples of Mexico and Central America. Psilocyn and psilocybin are Schedule I hallucinogens.

1.2 More information is available through the "Drug Identification Bible".

1.3 The Weber Test for the Presence of Psilocyn in Mushrooms", Garrette, Siemens, and Gaskill, NEAFS vol. XVIII, No.1, 1993.

2.0 Scope

2.1 The following procedures are used to identify psilocyn and/or psilocybin from mushrooms.

3.0 Equipment/Reagents

3.1 A GC/MS and appropriate analytical software. Reference AM #3.

3.2 ACS grade, or better, solvents: methanol, acetone, chloroform.

3.3 Fast Blue BB, or B salt.

3.4 Deionized/distilled water.

3.5 Hydrochloric and acetic acid.

3.6 Sodium bicarbonate.

4.0 Procedure

4.1 Color Spot Test

"Weber test".

4 .1.1 Add sample to well of spot plate after the addition of a Fast blue BB, or B, solution. Should turn orange-red within a couple of minutes if psilocin/psilocybin is present.

4.1.2 Remove some of the liquid to another well and then add a drop of concentrated HCl. A positive test is one that turns a blue-green color.

4.1.3 Negative and positive controls need to be run with each batch, and the results documented in the case notes

4.2 GC/MS Sample Preparation and Analysis

4.2.1 Extraction.

4.2.1.1 Extract with just enough methanol to cover sample. ***Note*** At this stage the methanolic extract may be injected into the GC/MS.

4.2.1.2 Centrifuge and decant solution into clean test tube. Cap and place into freezer until cold.

4.2.1.3 Remove from freezer and immediately add equal volume of cold acetone and mix.

4.2.1.4 Centrifuge, decant, and if necessary concentrate the supernatant.

4.2.2 Acid base extraction

4.2.2.1 Grind 1g of sample, add 10% acetic acid, grind, add 6ml water, grind to a slurry.

4.2.2.2 Add chloroform, mix and centrifuge/separate layers. Discard chloroform layer.

4.2.2.3 Slowly add Sodium Bicarbonate to pH 8-8.5.

4.2.2.4 Extract with chloroform, discard aqueous layer. Repeat with a 2% sodium bicarbonate solution.

4.2.2.5 Analyze on a GC/MS.

4.2.3 Analysis.

4.2.3.1 Run samples on GC/MS using a split or splitless data acquisition method depending on the sensitivity of the instrument.

4.2.3.2 Compare with a standard of either psilocyn or psilocybin. **NOTE** psilocybin breaks down into psilocyn in the hot injection port of a GC.

4.2.4 Conclusions and Reporting.

4.2.4.1 Confirmation. The retention time must be within 0.040 min of a valid scan of the standard and the MS spectra must match. If both conditions are satisfied then confirmation can be reported as "psilocyn and/or psilocybin (CI)".

4.2.4.2 If THC is detected in the GC/MS run then a second non-GC/MS test must be performed in order to confirm the THC.

AM #9 Lysergic Acid Diethylamide (LSD)

1.0 Background/References

1.1 LSD was originally synthesized from lysergic acid found in the fungus *claviceps purpurea.* Street LSD is found most often on blotter paper. It is also found on sugar cubes, candies like "Sweet Tarts", gelatin squares called windowpanes, and on small pills called microdots. It breaks down in the presence of light and heat, because of this the samples are often found wrapped in metal foil.

2.0 Scope

2.1 The following analytical procedures are used to confirm the presence of lysergic acid diethylamide (LSD).

3.0 Equipment/Reagents

3.1 A GC/MS and appropriate analytical software. Reference AM #3.

3.2 Ultraviolet light source.

3.3 Thin Layer Chromatography (TLC) plates and tank.

3.4 ACS grade, or better, solvents.

3.5 Distilled or deionized water.

3.6 $NaHCO_3$ or Na_2CO_3

3.7 The recipe for T1 is 7drops of ammonium hydroxide per 10ml of methanol.

3.8 The ratio of chloroform to methanol is 9/1.

3.9 p-DMAB is 1gram of p'dimethylaminobenzaldehyde in100ml of ethanol and 10ml of conc. hydrochloric acid.

4.0 Procedure

4.1 Ultraviolet (UV) Test

Although by no means definitive, this test can be used as a presumptive test. Place the evidence under the UV. The suspected LSD should glow a light violet-blue. This test is especially useful in identifying which side of a sugar cube, or candy, has been spiked with LSD. It is common for white paper to reflect the UV and appear violet even without LSD.

4.2 GC/MS Sample Preparation and Analysis

4.2.1 Sample preparation.

4.2.1.1 As with all GC analyses it may be necessary to concentrate the extracts from either of the following methods; this is done by blowing a stream of air, or other suitable gas, over the top of the solvent. Do not heat!

4.2.1.2 "Window panes", blotter paper, and pulverized microdots can be extracted using a basic extraction or directly with methanol. Place sample in a test tube and add just enough methanol to cover sample. Shake and then let soak for at least an hour. Microdots should soak overnight if possible. Centrifuge if necessary and analyze.

4.2.1.3 Sugar cubes, "Sweet Tarts" or other candy. Check under UV to find the side that is suspected of being spiked. Scrape off upper layer until up to one half of the sample, has been used. Dissolve in water and make basic. Extract with a minimal amount of chloroform. Analyze on GC/MS. Using the extraction procedure in 4.3.2, without the derivatizing agent, also works well.

4.2.1.4 Aqueous samples should be extracted using base and a minimal amount of chloroform.

4.2.2 Analysis

4.2.2.1 Due to the typically dilute nature of LSD samples, the GC may need to be set to splitless mode. The injector liner may have to be changed to a splitless model depending on the sensitivity of the particular MS being used. The retention time for LSD is concentration dependent. A series of standards of varying concentrations may have to be run in order to achieve the standard 0.040 minute retention time window.

4.3 TMS Derivative

At times, it may be necessary to derivatize weak LSD samples. The following is a summary of one possible method.

4.3.1 Reagents

4.3.1.1 Ammonium hydroxide (NH_4OH)

4.3.1.2 Methylene chloride, chloroform, or ethyl ether as solvents

4.3.1.3 MSTFA N-Methyl-N-trimethylsilyl-trifluoroacetamide

4.3.1.4 BSTFA bis(trimethylsilyl)trifluoroacetamide

4.3.2 Procedure

Place sample in concentrated NH_4OH and let soak for at least ten minutes. Add 200ul of solvent and extract. Separate and evaporate the solvent. Add 30-200ul of either MSTFA or BSTFA. Analyze on the GC/MS looking for the TMS derivative and comparing it to a derivatized standard.

4.4 TLC Analysis

A T1 system followed by p-DMAB color development works well for LSD. Other appropriate solvent systems, such as chloroform/methanol and acetone, may also be used. After the plate has been spotted with the sample extract, blank, and a standard, and the solvent has risen at least three quarters of the way up, remove the plate, dry, and then develop with p-DMAB. A purple color should develop with LSD.

4.4 Color Spot Tests

4.4.1 Marquis, grey color

4.4.2 p-DMAB, purple violet color

AM #10 GHB, GBL, and 1,4,BD

1.0 Background/References

GHB (gamma-hydroxybutyrate) is a controlled substance in Idaho while its precursors GBL (gamma- butyrolactone) and 1,4 Butanediol (1,4 BD) are not. This is problematic in that the interconversion of GBL to GHB is simply pH dependent and 1,4 BD is converted to GHB in the body. In aqueous solutions GHB and GBL will exist in equilibrium, the relative concentrations of each are also pH dependent.

The following analytical scheme was developed to separate and identify GHB, GBL, and 1,4 BD while ensuring that GHB is not produced during the process.

This method was based on a procedure found in "Microgram, Vol XXXV, No.1 January 2002".

2.0 Scope

2.1 The following analytical procedures are used to confirm the presence of GHB and its related analogs in samples.

3.0 Equipment/Reagents

3.1 A GC/MS and appropriate analytical software. Reference AM# 3.

3.2 FTIR and appropriate analytical software. Reference AM# 2.

3.3 pH paper

3.4 ACS grade, or better, chloroform, ethyl acetate, methanol, and ethanol.

3.5 H_2SO_4, BSTFA (with 1% TMCS) a TMS derivatizing reagent. ***Note*** the BSTFA is available premixed from Cerilliant Corporation.

3.6 Distilled or deionized water.

3.7 Bromocresol green, methyl orange, dextrose, aniline hydrochloride, sodium hydroxide. ***Note*, aniline is acutely toxic handle with care.**

4.0 Procedure

4.1 Screening Tests

4.1.1 Color Spot Test

A mixture of Bromocresol green, Methyl orange, and Schweppes reagents are tested with samples. A positive reaction for the presence of GHB is one that turns green.

4.1.1.1 Bromocresol green

Mix 0.03g bromocresol green in 100 mL of 4:1 methanol: water. Adjust to pH 7 with NaOH.

4.1.1.2 Methyl Orange

Mix 0.01g of methyl orange in 100 mL of methanol. Adjust pH to 7.

4.1.1.3 Modified Schweppes

4.1.1.3.1 Solution A: mix 2.0g dextrose in 20 mL of water.

4.1.1.3.2 Solution B: mix 2.4g aniline hydrochloride in 20 mL of ethanol.

Mix solution A & B and dilute to 80 mL with methanol.

4.1.1.4 For the final color reagent mix Bromocresol green solution with the Methyl Orange solution in a 1:1 ratio. Add 3 parts of this combined solution to one part of the Schweppes reagent.

4.1.2 Physical tests

4.1.2.1 Pure GBL and 1,4 BD are viscous liquids at room temperature. 1,4-BD will solidify when placed in a refrigerator (approximately 4°C) while GBL will not.

4.1.2.2 GBL is soluble in chloroform and 1,4 BD is not.

4.1.3 GC/MS

Add concentrated Sulfuric acid to aqueous sample, extract with chloroform and analyze (GC/MS). If GBL is detected then proceed with confirmational GC/MS.

4.2 GC/MS Sample Preparation and Analysis

GHB cannot be analyzed directly on a GC/MS as it will convert to GBL in the heated injector port. GHB must be derivatized with BSTFA before injection.

4.2.1 Butanediol

4.2.1.1 If pure 1,4 BD is suspected then dilute with methanol and inject into GC/MS.

4.2.1.2 In aqueous samples, if the concentrations of 1,4 BD are high enough, then the 1,4 BD may be observed in a chloroform extract.

4.2.1.3 Dry down sample, add methanol, and analyze.

4.2.1.4 1,4 BD will derivatize with BSTFA as per 4.2.2.3.

4.2.2 GHB

4.2.2.1 Extract aqueous samples with chloroform, discard chloroform layer.

4.2.2.2 Dry down aqueous layer with nitrogen or dry air. Sample can be warmed to expedite drying as long as the temperature remains below 60 C.

4.2.2.3 Once sample is completely dry then add 100-200 ul of BSTFA. Cap sample and heat at 60-70C for 15-20 minutes.

4.2.2.4 Add ethyl acetate and analyze on GC/MS.

4.3 FTIR

Aqueous samples appropriate for FTIR are defined as clear, colorless liquids that appear to be water. This doesn't include sodas, sport drinks, etc.

4.3.1 1,4-BD.

If suspected to be pure, run as a liquid sample, i.e. liquid cell, salt windows Gemini, ATR etc.

4.3.2 GBL

4.3.2.1 If pure then analyze as a liquid.

4.3.2.2 If aqueous, extract with chloroform. Discard aqueous layer. Evaporate off chloroform and run as a liquid.

4.3.3 GHB

4.3.3.1 If solid, analyze via the ATR or as a KBr pellet.

4.3.3.2 If aqueous, extract with chloroform. Discard chloroform layer. Evaporate to dryness and run via the ATR or as a KBr pellet.

4.4 Scheme

4.4.1 Solids

4.4.1.1 Run color test per section 4.1.1 of this method.

4.4.1.2 If color test is negative, dissolve in Methanol and analyze on GC/MS.

4.4.1.3 If color test is positive, skip to 4.4.1.5.

4.4.1.4 If GC/MS is negative then analysis is complete.

4.4.1.5 If GC/MS has GBL then derivatize original sample with BSTFA and analyze on GC/MS as per sections 4.2.2.3 and 4.2.2.4. Or run sample on FTIR.

4.4.2 Clear, thick liquids

4.4.2.1 Place 1-5 mls of the sample in the freezer for fifteen minutes. If it solidifies, extract with methanol and analyze with GC/MS.

4.4.2.2 If results indicate the presence of GBL proceed to section 4.4.3.4 and 4.4.3.5.

4.4.2.3 If sample remains a liquid go to section 4.4.3.

4.4.3 Aqueous samples

4.4.3.1 Perform color test per 4.1.1.

4.4.3.2 Acidify a portion of the sample with concentrated H_2SO_4 and extract with chloroform. Analyze the chloroform layer with GC/MS. If results are negative for GBL then proceed with section 4.4.3.3. If GBL is present then skip to section 4.4.3.4. If 1,4 BD is present then report.

4.4.3.3 If results from 4.4.3.2 indicate the presence of 1,4 BD then report. If results were negative then take a portion of original sample and dry down with nitrogen/air and heat (approximately 60C). Extract with methanol and analyze with GC/MS.

4.4.3.4 Take a portion of original sample extract with chloroform. Analyze chloroform layer with GC/MS. Report GBL if found.

4.4.3.5 Take aqueous layer from 4.4.3.4 and analyze using sections 4.2.2.2 through 4.2.2.4.

AM #11 Iodine

1.0 Background/References

1.1 Iodine is one of the essential ingredients in the production of methamphetamine using the ephedrine/pseudoephedrine/HI reduction method. The following methods, when used in combination, can be used to confirm the presence of iodine in samples typically found at clandestine laboratories.

1.2 A full copy of the Leuco Crystal Violet method can be found in Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998, Method 4500-I B.

2.0 Scope

2.1 The primary test is the Leuco Crystal Violet method. By itself this test cannot be used to confirm the presence of iodine due to the possible false positive reaction to oxides of manganese (MnO_2 and MnO_4). By using any of the additional tests the presence of the interferences is eliminated thus confirming iodine.

3.0 Equipment/Reagents

3.1 Equipment

3.1.1 Flasks and Stoppers

3.1.2 Pipets

3.1.3 pH test strips

3.1.4 Starch paper

3.2 Reagents. All reagent solutions can be scaled up or down from the following recipes. All water is deionized or distilled.

3.2.1 Stock iodine standard. Dissolve approximately 1.3g of KI into 1 L of water.

3.2.2 Citric Buffer

3.2.2.1 Citric acid. Dissolve 182.2g $C_6H_8O_7$ or 210.2g $C_6H_8O_7.H_{19}$ into 1 liter of water.

3.2.2.2 Ammonium hydroxide 2N. Dilute 131ml of conc. NH_4OH to 1L with water.

3.2.2.3 Final Buffer. Mix 350ml 2N NH_4OH to 670ml citric acid. Add 80g ammonium dihydrogen phosphate ($NH_4H_2PO_4$) stir to dissolve.

3.2.3 Leuco crystal violet indicator

3.2.3.1 Add 200ml water, 3.2ml H_2SO_4, and 1.5g 4,4',4'=-methylidynetris (N,N-dimethylaniline) ** to a 1L brown glass bottle. Mix upon each addition. **** NOTE**** AKA Leuco crystal violet.

3.2.3.2 Dissolve 2.5g mercuric chloride ($HgCl_2$) into 800ml water. ***Note*** Mercuric chloride is very toxic, avoid skin contact and inhalation.

3.2.3.3 Add $HgCl_2$ solution to Leuco crystal violet. Adjust pH to less than 1.5 with H_2SO_4 if necessary. Store, away from light.

3.2.4 Oxone. Potassium peroxymonosulfate ($KHSO_5$). Dissolve 1.5 g into 1L of water.

4.0 Procedure

4.1 *Procedure for Leuco Crystal Violet*

The goal of this test is the identification of iodine and not quantification; the following procedure has been condensed from the original. The following recipe is based on a final volume of 100 ml for both standard and sample solutions. Using the same proportion of reagents the volume can be successfully reduced. A test is considered positive if a violet color is developed. As with all procedures a blank and a standard are run with every batch.

4.1.1 Standard preparation.

4.1.1.1 Add 0.25 ml of iodine standard to a 100 ml flask. Dilute with 50 to 75 mL of water.

4.1.1.2 Add 1ml citric buffer and 0.5ml $KHSO_5$ solution. Mix and let stand one minute.

4.1.1.3 Add 1ml Leuco violet indicator, mix, and QS to 100 ml.

4.1.1.4 Color will often develop immediately. If not wait up to five minutes.

4.1.2 Blank preparation. Substitute deionized water for the standard and proceed with 4.1.1

4.1.3 Sample Preparation. The most difficult part of this analysis is judging how much sample to use. It is easy to use too much sample. If this occurs, a light blue-green- yellow color will develop instead of the expected violet.

4.1.3.1 Solid samples. Place a small piece of sample in a flask. Dissolve with 50 ml of water. Wait approximately one minute. Sample does not need to be completely dissolved. Proceed with 4.1.1 substituting the standard with the dissolved sample.

4.1.3.2 Liquids. Place a small amount of sample into a flask. Dilute with 50 to 75ml of water. The color of the sample solution at this point should be a very light yellow. Proceed with 4.1.1 substituting the standard with the diluted sample.

4.2 Complementary Methods for the Detection of Iodine

4.2.1 Heat. When a capped test tube containing solid iodine is subjected to moderate heating, violet fumes are created. Condensation of the fumes into shiny grey crystals at the cool top of the test tube will often be observed. The oxides of manganese that interfere with the Leuco crystal violet method do not produce this effect. As with all experiments involving heat this test should be done in a hood.

4.2.2 Starch Test. A liquid iodine solution when added to starch paper produces a blue-black stain, solutions made from the oxides of manganese do not.

4.2.3 Hexane Color Test. Hexane turns to a violet color when added to an iodine solution. Solutions containing oxides of manganese do not produce this effect.

4.2.4 pH Shift. Add sample to water and check pH, it should be neutral. Add red phosphorus and let stand. Check for a drop in pH (<2).

AM #12 Phosphorus

1.0 Background/References

The following method is used to identify elemental phosphorus. Phosphorus is typically found at clandestine methamphetamine laboratories. Although white phosphorus may be found, red phosphorus is most often encountered due in large part to its greater accessibility.

Method published in *"Clandestine Laboratory Investigating Chemists Journal, Vol. 10, #3".*

2.0 Scope

2.1 The following GC/MS analytical procedure is used to identify the presence of phosphorus.

3.0 Equipment/Reagents

3.1 A GC/MS and appropriate analytical software. Reference AM #3.

3.2 Test tube and holder

3.3 Bunsen burner or hand held propane torch

3.4 Chloroform ACS grade.

4.0 Procedure

4.1 This test is based on the fact that white phosphorus is soluble in chloroform while red phosphorus is not. Being soluble allows white phosphorus to be injected into a GC/MS.

4.1.1 Conversion of red to white phosphorus.

4.1.1.1 Take approximately 0.1g of red phosphorus into a test tube and heat with a Bunsen burner until the red phosphorus starts to emit yellow-white fumes signaling the conversion.

4.1.1.2 Remove from heat and immediately add chloroform. If white phosphorus is present then the above step is omitted.

***NOTE** Caution, the chloroform will boil and spit when added to test tube. The white phosphorus may ignite upon exposure to air. This part of the procedure **MUST** be done in a hood.

4.1.2 Analyze the extract on the GC/MS. Confirmation is achieved when the presence of the P2, P3, P4 ions are detected (MW 62, 93, 124 respectively) and compared to a standard.

4.2 Conclusions

The GC/MS procedure by itself can give a positive confirmation.